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Rapid Actions of Vitamin D Compounds

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Abstract The rapid actions of vitamin D compounds are surveyed in a variety of target tissues, including intestine, muscle, bone, hepatocytes, fibroblasts, HL-60 cells, kidney, mammary gland, and parathyroid. Evidence for non-nuclear receptors vs. membranophilic effects is discussed, followed by a consideration of signal transduction mechanisms *including* steroid hormone activated Ca^{2+} channels, phospholipid metabolism, protein kinases, and the role of G-proteins.

Key words: steroid hormones, non-genomic mechanisms, signal transduction, non-nuclear receptors, membranophilic effects

Vitamin D_3, acting through its daughter metabolite 1,25-dihydroxyvitamin D_3, [1,25$(OH)_2D_3$], is the major regulator of calcium homeostasis, and it is now considered to be a true steroid hormone acting via intranuclear receptor binding and regulation of gene transcription [1]. The now classical receptor-mediated pathway of 1,25$(OH)_2D_3$ action is not sufficient to account for all the known effects of the seco-steroid. In the last 11 years, evidence has emerged indicating that in addition to the nuclear receptor-mediated mechanism, vitamin D metabolites may also operate through other mechanisms, particularly through an effect on plasma membrane. These non-genomic actions of 1,25$(OH)_2D_3$ are characterized by their instantaneous onset or a very short latency, as well as insensitivity to inhibitors of RNA and protein synthesis.

A SURVEY OF TISSUE/CELL TYPES AND THE RAPID EFFECTS THEY EXHIBIT

Intestine

As a major target tissue of the hormonally active metabolite 1,25$(OH)_2D_3$, work on the intestine has provided the first observations on rapid actions of the seco-steroid. In 1979, Bachelet et al. [2] reported the stimulation of brush border alkaline phosphatase activity after a 30 min ex *vivo perfusion of* rat intestine with 1,25$(OH)_2D_3$. More recently, a 10 min stimulation has been reported [3]. The authors postulated that the increase was independent of de novo protein synthesis, and may indeed reflect a segment of the vesicular calcium transport pathway [4,5]. Using isolated rat enterocytes, it has also been demonstrated that 1,25$(OH)_2D_3$ rapidly stimulates calcium uptake [4,6], and lysosomal enzyme release [4]. The latter phenomenon is believed to represent calcium transport through vesicular pathways [7–9].

The duodenal loop of normal, vitamin D-replete chicks has yielded the best evidence that net transport of calcium is acutely stimulated by 1,25$(OH)_2D_3$ [cf. 9]. This functional physiological response to the seco-steroid hormone has also made it an attractive system for studies on signal transduction mechanisms. In addition, Vesley and Juan [10] have reported 1,25$(OH)_2D_3$-augmented guanylate cyclase activity in intestine within 10 min of treatment with exogenous hormone. Although enhanced cGMP levels were not correlated with changes in Ca^{2+} transport, the seco-steroid hormone evokes similar phenomena in other tissues (see below).

Muscle

Vitamin D_3 metabolites are important regulators of muscle function [11]. In vitro studies performed in skeletal and cardiac muscle from vitamin D-deficient chicks have documented an acute effect (1–15 min) of 1,25$(OH)_2D_3$ on Ca^{2+} uptake [12,13]. The hormone acts in a dose-dependent fashion and its effects could be de-

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tected at physiologically relevant doses (10^{-10} to 10^{-11} M). In contrast $24,25(OH)_2D_3$, $25(OH)_2D_3$, and vitamin D_3 are much less potent. The fast effects of $1,25(OH)_2D_3$ are unaffected by inhibitors of RNA and protein synthesis but can be suppressed by Ca^{2+}-channel antagonists, indicating that the hormone can act directly at the muscle membrane level affecting Ca^{2+} entry into the cell. The effects of the hormone on muscle Ca^{2+} influx are accompanied by a stimulation of microsomal membrane protein phosphorylation [14,15]. These changes are also evidenced following direct exposure of isolated muscle microsomes to $1,25(OH)_2D_3$, supporting a direct action of the hormone on muscle membranes. The early action of $1,25(OH)_2D_3$ on skeletal muscle Ca^{2+} uptake is paralleled by a transfer of calmodulin from cytosol to membranes [16] and has been associated with increased ability of two microsomal proteins of 28 and 30 KDa to bind calmodulin [17,18]. The fact that forskolin mimics the effect of the hormone on calmodulin redistribution and binding ability and that these actions are abolished by protein kinase A inhibitors and phosphatases [18] indicates that $1,25(OH)_2D_3$ affects calmodulin binding ability through cAMP-dependent phosphorylation. Calmodulin antagonists are able to inhibit $1,25(OH)_2D_3$-dependent Ca^{2+} uptake and reverse the effect of the hormone on calmodulin distribution.

Bone

In the early 1980s Eilam et al. [19] found that both $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ increase the initial rate of Ca^{2+} influx in primary cultures of rat bone cells through a mechanism insensitive to the effects of protein synthesis inhibitors. More recently it has been reported that in primary cultures of mouse osteoblasts, physiological concentrations of $1,25(OH)_2D_3$, $24,25(OH)_2D_3$ and $25(OH)_2D_3$ rapidly induce (within 15 sec) significant increases in $[Ca^{2+}]_i$ [20]. The response to $1,25(OH)_2D_3$ is apparently dependent on Ca^{2+} influx, whereas $25(OH)_2D_3$ and $24,25(OH)_2D_3$ mobilize Ca^{2+} from intracellular stores as shown by blockade experiments with Ca^{2+}-channel antagonists and drugs which alter the Ca^{2+} sequestration by intracellular organelles. Transient increases in $[Ca^{2+}]_i$ are also observed in the osteoblastic cell line MC3T3-E1 [21], and in single osteogenic sarcoma cells (ROS 17/2.8). In the latter cells, the effect is entirely dependent on extracellular calcium at low hormone levels [22], while at higher doses (10^{-8} to 10^{-7} M), $1,25(OH)_2D_3$-dependent elevation of $[Ca^{2+}]_i$ is due to both influx of extracellular Ca^{2+} and release of Ca^{2+} from intracellular stores. In these cells, $25(OH)_2D_3$ reproduces the effects of $1,25(OH)_2D_3$ on intracellular Ca^{2+} with equal potency and similar responses, whereas $24,25(OH)_2D_3$, 1- alpha-$(OH)D_3$, and 22-oxa-$1,25(OH)_2D_3$ are not effective. Patch-clamp studies confirmed that in these cells $1,25(OH)_2D_3$ acts as a potent modulator of L-type Ca^{2+}-channel function [23]. The effect of the hormone on Ca^{2+} currents is specific since $25(OH)D_3$ is effective only at concentrations of 10^{-9} M and greater and $24,25(OH)_2D_3$ exhibits less potent stimulatory effects on Ca^{2+} currents.

Hepatocytes

Rat liver cells have been reported to respond to $1,25(OH)_2D_3$ within 5 min by the criterion of enhanced cytosolic calcium [24]. Fluorescently determined calcium levels were found to increase in response to the seco-steroid hormone whether or not extracellular calcium was present. Moreover, the response was highly selective for $1,25(OH)_2D_3$, relative to other metabolites and analogues tested.

Fibroblasts

Cultured human fibroblasts have been found to respond within one minute to low levels of $1,25(OH)_2D_3$ with dramatic increases in intracellular cGMP [25]. Although the ultimate effect of the seco-steroid hormone on fibroblasts is unknown, it is tempting to speculate that the agonist might have an effect on the lysosomal calcium transport system in such cells [26].

HL-60 Cells

The promyelocytic cell line HL-60 can be induced to differentiate over a period of days by the seco-steroid hormone, while changes in cytosolic calcium occur much more rapidly. A $1,25(OH)_2D_3$-mediated augmentation in cytosolic calcium occurs within 5 min of addition of exogenous hormone [27], whereas a calcium efflux has been reported to occur after a 2–4 h treatment, even in the presence of protein- or RNA synthesis inhibitors [28].

Kidney

In kidney $1,25(OH)_2D_3$ is known to stimulate the reabsorption of both calcium and phosphate.

Using purified brush border membrane vesicles from renal tissue, Kurnik et al. [29] reported a hormone-mediated alteration in phospholipid composition and phosphate transport. Alterations in membrane fluidity, and hence phosphate transporter activity were also reported by Suzuki et al. [30]. Edelman et al. [31] reported that $1,25(OH)_2D_3$ inactivated calcium-dependent potassium channels at the apical cell membrane of the proximal tubule, although the phenomenon could not at the time be related to a particular physiological mechanism. The hormone is also able to increase guanylate cyclase activity and cGMP production within 10 min of exposure [10].

Mammary Gland

The differentiated epithelial cells of mammary gland also transport calcium for the production of milk. It is not surprising that $1,25(OH)_2D_3$ stimulates calcium uptake in explants. Augmented uptake was observed within 30 min of hormone addition and was unaffected by the presence of actinomycin D [32].

HORMONE EFFECTOR MECHANISMS AND TISSUES IN WHICH THEY OCCUR

Non-Nuclear Receptors vs. Membranophilic Effects

In 1979, Norman and Ross [33] postulated that in addition to the nuclear actions of $1,25(OH)_2D_3$ in regulating gene expression, the seco-steroid might insert directly into the lipid bilayer to exert a "membranophilic" effect. Completely independent of this postulate, Nemere and Szego [4] proposed that $1,25(OH)_2D_3$ might exert its rapid actions through a membrane-associated receptor, in analogy to peptide hormone action.

Membranophilic effects have been postulated by Schwartz et al. [34] to explain their observations that two metabolites, $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$, exert direct effects on the alkaline phosphatase and phospholipase-A_2 specific activities in membranes of growth zone and resting zone chondrocytes. A similar mechanism has also been invoked as the basis for the observed $1,25(OH)_2D_3$-mediated changes in renal brush border phosphate uptake [29,30] and membrane potential effects in proximal tubule [31].

Receptors near, but not in the membrane apparently mediate the actions of $1,25(OH)_2D_3$ in fibroblasts [25]. As recently reviewed [9], a variety of tissues contain membrane receptors for steroid hormones, and interaction of the two molecules often results in calcium uptake by the target tissue. This type of effector-transduction mechanism has been postulated to account for $1,25(OH)_2D_3$-activated calcium channels in intestine [35], enterocyte phosphoinositide metabolism [26], and activation of intestinal protein kinases [37].

In both osteoblasts [38] and intestine (Zhou et al., manuscript submitted), receptor specificities for non-nuclear effects appear to be different from ligand preferences for the nuclear receptor. In osteoblasts, the analogs 25(OH)-16-ene-23-yne-D_3 and 25-(OH)-23-yne D_3 are potent calcium channel activators and bind poorly to the nuclear receptor [38]. In chick intestine, these analogs also bind poorly to the nuclear receptor but are relatively efficient in stimulating transcaltachia, the rapid hormonal stimulation of calcium transport (Zhou et al., manuscript submitted). Thus one of the priorities of unravelling the mechanism of $1,25(OH)_2D_3$-mediated rapid effects will be to isolate and characterize non-nuclear receptors.

Signal Transduction Mechanisms

An increasing body of evidence indicates that $1,25(OH)_2D_3$ exerts early physiological effects in several cell systems by activation of signal transduction mechanisms similar to those elicited by peptide hormones after interactions with membrane receptors.

Ca^{2+}-channels. Ca^{2+}-channels are a major route by which Ca^{2+} ions enter the cell to regulate some of their fundamental functions. The observation that $1,25(OH)_2D_3$ increases dihydropyridine (DHP)-sensitive Ca^{2+} uptake in skeletal and cardiac muscle [12,13], transcellular Ca^{2+} transport in intestine [35] and Ca^{2+} influx in various cell types [20,22,39] suggests that the action of the hormone in those cells is mediated by the activation of voltage-operated Ca^{2+}-channels. However, the sterol is structurally different from DHP, therefore it is unlikely that it binds to regulatory sites on channel proteins. It is more likely that the rapid effects of the hormone are mediated indirectly by activation of second messenger systems. An important feature of voltage-operated Ca^{2+} channels is that their activity is regulated by phosphorylation [40]. Phosphorylation of the channel (or a closely

associated protein) leads to an increase in the probability of channel opening.

There is evidence indicating that protein kinases A and C mediate $1,25(OH)_2D_3$ activation of Ca^{2+}-channels in muscle and intestine [13–15,37]. The selective inhibition of $1,25(OH)_2D_3$-dependent Ca^{2+} transport by Ca^{2+}-channel antagonists and inhibitors of kinases A and C, and the similarity of $1,25(OH)_2D_3$ responses to agonists of these enzymes, suggests that phosphorylation is the key event by which the hormone modulates Ca^{2+}-channels in muscle and intestinal tissue. Furthermore, exposure of skeletal and heart muscle to $1,25(OH)_2D_3$ for intervals similar to those at which the rapid Ca^{2+} uptake response to the hormone is elicited, stimulates the phosphorylation of membrane proteins [13,14]. In skeletal muscle, as a consequence of $1,25(OH)_2D_3$ action, a complex interplay between the two major transmembrane signalling systems is observed [41], suggesting that protein kinase C-dependent phosphorylation affects in some way the activity of the adenylate cyclase system and/or cAMP phosphodiesterases. Thus, modulation of DHP-sensitive Ca^{2+}-channels is likely to be one of the primary mechanisms underlaying $1,25(OH)_2D_3$-mediated regulation of intracellular calcium.

G-proteins. Ca^{2+}-channel function is known to be regulated by guanine nucleotide-binding proteins (G proteins) either directly or indirectly through G protein interaction with specific effectors that in turn activate second messengers such as cAMP and IP_3. The involvement of G proteins in $1,25(OH)_2D_3$ mode of action has been recently suggested in hepatocytes [24], and skeletal muscle [42]. In skeletal muscle, a number of observations lead the authors to propose that an interaction with an inhibitory G protein coupled to adenylate cyclase may be part of the mechanism by which $1,25(OH)_2D_3$ increases Ca^{2+} uptake through regulation of Ca^{2+}-channel gating by a cAMP-dependent pathway in this tissue.

Phospholipid metabolism. In enterocytes [36,43], keratinocytes [44,45], osteoblasts [21], and parathyroid (Bourdeau et al., 1990 [46]) a breakdown of membrane phosphoinositides is elicited by $1,25(OH)_2D_3$ in a concentration dependent manner to generate inositol triphosphate (IP_3; a Ca^{2+} mobilizer) and diacyl glycerol (DAG; a protein kinase C activator) within seconds to minutes of exposure to hormone. In contrast, $1,25(OH)_2D_3$ does not modify the levels of IP_3 in CH4C1 pituitary cells [47]. In enterocytes [43], as a consequence of the rise in DAG content and intracellular calcium, $1,25(OH)_2D_3$ transiently activated protein kinase C, a known regulator of proliferation and differentiation in many cell systems. In various cell types, the rise in IP_3 coincided with the initial rise in $[Ca^{2+}]_i$ suggesting that it may be responsible for the calcium response observed in these cells.

In enterocytes from young rats, at a time when specific binding sites for $1,25(OH)_2D_3$ can not be detected, $1,25(OH)_2D_3$ failed to raise IP_3 and DAG levels [36]. This observation suggests that the presence of functional $1,25(OH)_2D_3$ receptors may be a prerequisite for the early action of the hormone on the phosphinositide pathway.

In hepatocytes, $1,25(OH)_2D_3$-induced changes in cytosolic calcium were also found to be related to phosphatidylinositol metabolism, but the active agent was lysophosphatidylinositol, which in turn utilized a pertussis toxin-sensitive G protein for signal transduction [24].

CONCLUSIONS

Aside from characterization of non-nuclear receptors, future work should address how the various signal transduction mechanisms relate to the ultimate biological response. In many tissues, the biological response remains to be identified; in intestine many of the signal transduction mechanisms can be related to the vesicular transport of calcium. Cyclic nucleotides, protein kinase activation, G-proteins, activation of Ca^{2+}-channels, and phospholipid metabolism have all been related to endocytosis, movement of vesicles along cytoskeletal elements, or exocytosis in a wide variety of cell types. Such regulatory systems for vesicular flow probably operate in intestine, as well as such tissues as parathyroid. In these endocrine cells $1,25(OH)_2D_3$ has been shown to enhance rapid calcium uptake and phospholipid metabolism [46]. Phosphoinositides have been reported to be associated with clathrin-coated vesicles in brain [48] and secretory granules in parathyroid tissue [49]. In hepatocytes $1,25(OH)_2D_3$ also stimulates lysophosphatidyl inositol formation [24], although a specific exocytotic event has yet to be identified. Calcium transporting tissues such as kidney and bone cells are likely also to use a vesicular pathway for movement of the divalent cation, and $1,25(OH)_2D_3$-regulated signal transduction mechanism might be geared toward this end. In other target tissues, such membrane/

cytoskeletal events might be secondary to the main biological response. In either case, study of the rapid actions of vitamin D compounds should enlarge our knowledge of steroid hormone actions.

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